cytosolic side of the membrane. Disruption of this electrochemical equilibrium causes V_m to become more positive or more negative relative to its resting state, referred to as ''depolarization'' or ''hyperpolarization,'' respectively. Changes in membrane potential have proven to be pivotal not only in normal cell cycle progression, but also in malignant transformation. Using polystyrene nanoparticles as a model system, we use a combination of fluorescence microscopy and flow cytometry to measure changes in membrane potential in response to nanoparticle binding to the plasma membrane. We find that cationic nanoparticles depolarize both CHO-K1 and HeLa cells. The cellular binding of anionic nanoparticles does not lead to a discernible trend in altered membrane potential. Maintenance of the resting membrane potential depends on the presence of two-pore-domain potassium ''leak'' channels, which allow for outward diffusion of potassium ions along their concentration gradient. Using an assay that tests the diffusion of ions through these potassium channels, we observe reduced permeability of the channels when cells are treated with nanoparticles. Based on a dynamical system model of the cell, we conclude that this loss of permeability likely results from physical blockage of the channel itself by the particle. Prevention of potassium ion efflux due to blocked channels causes accumulation of positive charge inside the cell, resulting in a depolarized membrane. By understanding the ways in which nanoparticles can be utilized to selectively generate cellular responses, we can begin to consider them as active species that may alter the very systems they are currently designed to probe.

935-Plat

Electro-Wetting of a Hydrophobic Gate in a Biomimetic Nanopore

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Nanopores in membranes have a range of potential applications. Biomimetic design of nanopores aims to mimic key functions of biological pores within a stable template structure. Molecular dynamics simulations have been used test whether a simple β-barrel protein nanopore can be modified to incorporate a hydrophobic barrier to permeation. Simulations have been used to evaluate functional properties of such nanopores, using water flux as a proxy for ionic conductance. Potential of mean force calculations have been used to calculate free energy landscapes for water and for ion permeation in pore models. These studies demonstrate that a hydrophobic barrier can indeed be designed into a β-barrel protein nanopore, and that the height of the barrier can be adjusted by modifying the number of consecutive rings of hydrophobic sidechains. A hydrophobic barrier prevents both water and ion permeation even though the pore is sterically unoccluded [1].

A clear prediction of the hydrophobic gating model is that of electro-wetting of the gate should occur, i.e. the pore can be functionally opened by applying a high transmembrane voltage. This has been seen experimentally in studies of hydrophobically-gated solid state nanopores [2]. We have explored electro-wetting of our model of a hydrophobic gate in a simple β-barrel protein nanopore using atomistic molecular dynamics simulations with either a constant field applied or with the recently developed 'computational electrophysiology' approach [3] to model a voltage difference across the pore and bilayer. The results of both methods demonstrate voltage-dependent de-wetting in these pores.

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936-Plat

Deformation of MCF-7 Cells in Micropores with Undulating Diameter

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Pores used in current resistive-pulse experiments were shown to measure size and surface charge of translocating objects. With the addition of the undulation of the pore opening diameter it is possible to simultaneously characterize the size and mechanical properties of the object that has been passed through the pore. Here we will discuss in detail the characterization of a cell's size and mechanical properties by examining the resistive-pulse of each individual cell. The cells translocated the pore electrokinetically and no external pressure difference was applied. Previous experiments with polystyrene and hydrogels particles as well as numerical modeling of electroosmotic fluid flow in our pores revealed existence of pressure drops along the pore axis. The local pressure gradients were predicted to deform biological cells even if the pore opening was larger than the cell at any axial position. Polystyrene particles suspended in a solution of HBSS and Tween 80 were first passed through single undulating micropores to measure their topography. MCF-7 cells were suspended in a solution of HBSS and pluronic, and passed through the same pores. Deformation of the cells was observed as a change of the relative amplitude of the pulse sub-peaks compared to the signature obtained with the hard polystyrene particles. Viability of the cells after their passage through the micropore was also established.

937-Plat

Fingerprinting Single Living Cells with Molecular Precision

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The secretome of a single living cell contains the totality of its secreted proteins,(1) and therefore can act as a fingerprint by which to identify cell type. Although between 10 % and 20 % of the human genome encodes proteins that are secreted, measuring the secretome from an individual living cell is challenging as the secreted proteins are present in vanishingly small concentrations due to the very large dilutions involved.

However, a nanopore is able to detect single proteins through the distinctive blockage profile that develops in the ionic conductance current when a protein passes through the nanopore.(2,3) Using a synthetic nanopore in a silicon membrane we investigate the distinctive blockage patterns, in essence the fingerprint, that arise from a single living cell. The cell is placed in proximity to the nanopore using optical tweezers and held stationary. The ionic conductance current is measured across the nanopore, and translocation events (distinct blockage currents) are observed and measured. When the events are plotted in scatter plots (as dwell time versus average blockage current) the distinct fingerprint of individual cells can be observed. For instance, lymphoma cells (U937) and breast cancer cells (MCF7) produce distinct event patterns that enable them to be distinguished. This shows for the first time cell identification based entirely on the secretome, measured using a simple, non-invasive, non-destructive nanopore.

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938-Plat

Mechanical Modulation of Enzyme Activity by Rationally Designed DNA Tweezers: From the Ensemble to the Single-Molecule Level

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Switchable nanomachines provide a platform to control dynamic functional states by altering distances at the nanoscale on demand. Recently, a tweezers-like DNA device was used to control the activity of an enzyme/cofactor pair juxtaposed on the two arms of the tweezers. Initial studies focused on bulk properties of the tweezers-mediated reactions, and hence lacked insight into the mechanism of enzymatic activation. Here, we used site-specific fluorophore labeling of the tweezers to monitor the arm-to-arm distance through single-molecule fluorescence resonance energy transfer (smFRET). Consistent with AFM measurements, smFRET showed that the tweezers only partially close in the proposed ''closed'' state and exhibit conformational sub-states. MD simulations showed bending and twisting of the tweezers arms, rationalizing the sub-states. Additionally, smFRET experiments on the isolated Holliday junction hinge suggested that the isomer resulting in the tightest closing of the tweezers (isomer-I) is relatively disfavored, further explaining the only partial closing. We rationally improved the closing by increasing the stem length of the DNA hairpin bridging and actuating the tweezers from 3 to 4 base pairs, and by redesigning Holliday junction(s) of the tweezers to favor the optimal isomer-I. The performance of the new tweezers was quantitatively assessed by juxtaposing glucose-6-phosphate dehydrogenase (G6pDH) with its cofactor NAD^+ on the tweezers arms and measuring the G6pDH activity through a coupled enzymatic cascade. Using our optimized tweezers, we were able to enhance the bulk activity of G6pDH upon tweezers closure to up to ~12-fold. Currently, we are exploring the tweezers-manipulated enzymatic reaction

at the single-molecule level. Our results suggest that G6pDH stochastically fluctuates between active—inactive states, favoring the active state upon closure of the tweezers. Our discovery may represent a general approach for refining nanodevices for advanced applications.

939-Plat
Regulation of Lipid Membrane Trafficking and Transmembrane Signaling by Graphene
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Cholesterol, a lipid molecule found ubiquitously in eukaryotic cells, plays a vital role in the integrity, dynamics, and trafficking of the lipid membrane, in addition to influencing many transmembrane proteins. However, the functionality of membrane cholesterol is far from clear, largely due to an inability to manipulate membrane cholesterol with high spatiotemporal precision. Popular tools like statins or methyl-β-cyclodextrin (MβCD) only lead to chronic and indiscriminative cholesterol reduction. Moreover, there is no selective approach to increase membrane cholesterol. Our recent work involving carbon nanomaterials provided an unexpected answer. Graphene, a one-atom thick carbon crystal, has been explored for biomedical applications because of its remarkable chemical and physical properties. Using in vitro and in vivo measurements, we have found that graphene selectively interacts with cholesterol. This enriches cholesterol at the plasma membrane, and thus enhances membrane lipid phase order, likely promoting the formation of cholesterol-rich lipid membrane nanodomains. Neurons grown on graphene exhibited presynaptic potentiation, specifically caused by a larger pool of releasable vesicles and an increase of fast recycling. By addition or depletion of membrane cholesterol, we found that the graphene-induced presynaptic enrichment of membrane cholesterol is necessary and sufficient to promote potentiation. In non-neuronal cells, graphene significantly elevates ATP-induced intracellular Ca^{2+}-signaling by promoting the activation of P2Y receptors, a group of GPCRs which are selectively responsive to extracellular ATP. Furthermore, we found that graphene enhances P2Y receptor signaling on the timescale of seconds, as rapidly as its effect on membrane packing. This then reveals an intriguing interaction between graphene and cholesterol, and its impact on plasma membrane structure, trafficking, and transmembrane proteins. Given the current challenges in manipulating membrane cholesterol, this graphene-cholesterol interaction will accelerate studies of membrane cholesterol function and broaden the biological application of carbon nanomaterials.

940-Plat
Development of a Fluorescence-Based Assay for Functional Studies of Transporter Proteins on the Single Molecule Level
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Regulated and selective transport of biomolecules across biological membranes is mediated by transporter proteins. These are essential in physiological processes ranging from electrical signaling in the nervous system to secretion of hormones and maintenance of electrochemical gradients. Though transporters are extensively studied, there are currently no techniques available to investigate their function at the single molecule level. We have developed a fluorescence-based assay to monitor thousands of individual nanoscale proteoliposomes in parallel by immobilizing them on functionalized glass surfaces (1-5).
We recently extended this assay to allow real-time observation of substrate translocation mediated by single transporters reconstituted into liposomes. Here we present the development of the assay including characterization and optimization of the most critical system components including: minimization of leakage, in situ real-time calibration of absolute transport rates and increase of photostability for long term recordings (~40min). We also present high-resolution single molecule activity recordings of the transporter protein, Arabidopsis thaliana H+-ATPase (AHA2).

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Symposium: Epigenetics

941-Symp
Cost and Precision in Small Gene Regulatory Networks
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Genes in early fly development are expressed with astonishing precision, despite the molecular noise intrinsic to all biochemical reactions. In an attempt to understand the regulatory mechanisms in this system, I will discuss the nature of hunchback mRNA expression based on analysis of live imaging experiments. Inspired by fly development, I will then discuss trade-offs that gene regulatory circuits have to face in order to precisely respond to signals in the presence of molecular noise.

942-Symp
Metaphase Chromatin Plates Explain the Structure and Physical Properties of Condensed Chromosomes
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Previous studies showed that during mitosis chromatin filaments are folded into multilayer plates (1). These structures can be self-assembled from chromatin fragments obtained by micrococcal nuclease digestion of metaphase chromosomes (2). Chromosomes of different animal and plant species show great differences in size (which are dependent on the amount of DNA that they contain), but in all cases chromosomes are elongated cylinders that have relatively similar shape proportions (the length to diameter ratio is approximately 13). It is possible to explain this morphology by considering that chromosomes are self-organizing supramolecular structures formed by stacked layers of planar chromatin having different nucleosome-nucleosome interaction energies in different regions (3). The nucleosomes in the periphery of the chromosome are less stabilized by the attractive interactions with other nucleosomes and this generates a surface potential that destabilizes the structure. Chromosomes are smooth cylinders because this morphology has a lower surface energy than structures having irregular surfaces. The symmetry breaking produced by the different values of the surface energies in the telomeres and in the lateral surface explains the elongated structure of the chromosomes. The results obtained by other authors in nanomechanical studies of chromatin and chromosome stretching have been used to test the proposed supramolecular structure. It is demonstrated quantitatively that internucleosome interactions between chromatin layers can justify the work required for elastic chromosome stretching. Chromosomes can be considered as hydrogels with a lamellar liquid crystal organization. The good mechanical properties of this structure may be useful for the maintenance of chromosome integrity during mitosis. Furthermore this chromatin organization avoids random entanglement of the extremely long genomic DNA molecules in chromosomes.
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943-Symp
Cooperativity and Supercoiling Modulate Functions of Human O^6-alkylguanine DNA Alkyltransferase
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Human cells contain DNA alkyltransferases that protect genomic integrity under normal conditions but also defend tumor cells against chemotherapeutic alkylating agents. Here we explore how structural features of the DNA substrate affect the binding and repair activities of the human O^6-alkylguanine-DNA alkyltransferase (AGT). In vitro, cooperative binding results in all-or-nothing association on short templates. A requirement for contact with 4 DNA base-pairs results in oscillation of average binding site size S_{app} and cooperativity factor w with template length. Models in which protein molecules overlap along the DNA contour predict that protein-protein contacts will be optimal when the DNA is torsionally relaxed. Supporting this prediction, topoisomerase assays show that AGT binding is accompanied by a